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Selective Modulation of Lipopolysaccharide-Induced Death and Cytokine Production by Various Muramyl Peptides

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Pretreatment of animals with the adjuvant muramyl dipeptide enhances both the production of circulating tumor necrosis factor and the sensitivity to the lethal effect of a lipopolysaccharide (LPS) challenge. The present study examined the capacity of various adjuvant muramyl dipeptide derivatives to potentiate responsiveness to LPS administration. Cytokine levels in serum were determined at various time intervals after LPS administration by bioassays and immunoassays; the cytokines examined were tumor necrosis factor, interleukin-1, interleukin-6, and gamma interferon. The time course of cytokine response was not modified by the pretreatment, but most of the levels were strongly enhanced. However, of the four compounds which were found to be potent priming agents, only two caused an increased sensitivity to LPS lethality, showing that elevated titers of cytokines in serum were not correlated with host sensitization. Interestingly, previous studies have shown that these two compounds also display neurobiological properties, implying a possible role of the central nervous system in LPS lethality. However, two hydrophilic derivatives with low activity as priming agents were capable of decreasing the toxicity of LPS when given after the challenge in galactosamine-sensitized mice. These results illustrate the diversity of responses elicited by immunological priming. They raise unanswered questions on the importance of endogenous mediators in the pathophysiological alterations during toxic shock.

Sequential cytokine induction by cells of the mononuclear phagocyte system has been found in vitro and in vivo following endotoxin stimulation. These mediators, especially tumor necrosis factor (TNF), interleukin-6 (IL-6), and IL-1, which are thought to be important as regulators of the immune system under physiologic conditions, can display toxic effects when produced in excess. There is evidence that they play a role in the pathologic manifestations of endotoxemia, although it is unlikely that any one of them alone could be responsible for death. TNF has been shown to be an important mediator of toxic shock, as demonstrated by the protective effect of passive immunization (4). Its role was later confirmed by the resistance to endotoxic shock of mice given soluble TNF receptor proteins (2, 21, 44) or made deficient for the 55-kDa TNF receptor (31). On the other hand, the protective role of IL-1 receptor antagonist (IL-1ra) has indicated that IL-1 can function as a mediator in toxic shock (1, 12) and antibodies against IL-6 or against leukemia inhibitory factor (LIF) have been shown to exert some protective effects against endotoxemia (6, 33, 49).

The lymphokine gamma interferon (IFN-γ) was first shown to be involved in the pathogenesis of a Shwartzman-like lethal response to lipopolysaccharide (LPS) in mice (5). More recently, anti-IFN-γ antibody has been shown to exert a protective effect against a single lethal dose of LPS either in unprimed mice (10, 17) or in *Mycobacterium bovis* BCG-infected animals with a targeted disruption of the IFN-γ receptor (20). During sensitization to LPS-induced death observed after an infectious challenge with various gram-positive or gram-negative bacteria, endogenous IFN-γ production appears as a key element in the complex process leading to hyperreactivity (reviewed in reference 13). In parallel, these animals are primed for enhanced cytokine release following LPS administration like those pretreated with IFN-γ (14, 17). However, the protective effect of anti-IFN-γ therapy against endotoxic shock is mediated by mechanisms other than modulation of monokine levels, since endogenous IFN-γ appears only after TNF levels in serum have already peaked (17). Furthermore, it has been reported that antibodies to IFN-γ failed to protect galactosamine-sensitized mice against the lethal effect of LPS (18) and that both the level and the time course of IFN-γ and TNF production were very similar in mice given toxic LPS and in those given nontoxic monophosphoryl lipid A (22).

Muramyl dipeptide (MDP) is a synthetic immunoadjuvant which can induce TNF, IL-1, and IL-6 mRNA accumulation and protein secretion in mouse macrophages (28). In vivo, MDP alone cannot produce detectable levels of cytokine bioactivity in the blood (43). However, in MDP-primed mice, a challenge with LPS results in elevated titers of circulating TNF, IL-1, or IL-6 and a significant increase in the toxic lethality of LPS (8, 40). Some differences have already been reported for certain MDP derivatives regarding both the toxic synergism with LPS and the priming effect for enhanced cytokine responses (8, 43).

In this report, we have confirmed that some immunoadjuvant-active MDP derivatives had the capacity to enhance the lethal effect of LPS whereas other selected analogs were devoid of such activity. The lack of toxic synergism between hydrophilic but not lipophilic MDP derivatives and LPS correlated with a lack of priming for enhanced cytokine induction. Moreover, we have shown that two water-soluble MDP analogs could reverse the lethal effect of LPS when given 1 h after the challenge.

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MATERIALS AND METHODS

Mice. Pathogen-free OF1 female mice (8 to 10 weeks old) were purchased from Iffa Credo, St-Germain sur l'Abresle, France, and C3H/HeJ mice were purchased from CSEAL, Orléans, France.

Reagents. MDP (*N*-acetylmuramyl [AcMur]-L-Ala-D-iGln), murabutide (AcMur-L-Ala-D-Gln-*n*-butyl ester), murametide (AcMur-L-Ala-D-Gln-α-methyl ester), MDP-GDP (MDP-1,2-dipalmitoyl-*sn*-glycerol) and its stereoisomer MDP(D,D)-GDP, and MDP(Thr)-GDP (AcMur-L-Thr-D-iGln-GDP) were provided by Vacsyn S.A., Paris, France. MDP hydrophilic derivatives (MDP, murabutide, and murametide) were directly dissolved in phosphate-buffered saline (PBS), whereas the lipophilic derivatives [MDP-GDP, MDP(D,D)-GDP, and MDP(Thr)-GDP] were heated at 56°C for a few minutes before being ground in a Potter tube to produce a homogeneous dispersion in PBS. *Salmonella enteritidis* LPS extracted by the phenol-water procedure was obtained from Difco Laboratories, Detroit, Mich., and suspended in PBS. D-Galactosamine was obtained from Sigma. BCG was kindly supplied by M. Gheorghiu, Pasteur Institute, Paris, France. The cytokines used to standardize the bioassays were as follows: recombinant murine TNF (rMuTNF) was a gift of W. Fiers, Ghent, Belgium; recombinant human IL-1β (rHuIL-1β) was kindly provided by D. Lando, Roussel-Uclaf; and rMuIL-6 was purchased from Genzyme, Cambridge, Mass. IL-1ra was generously supplied by R. C. Thompson, Synergen, Inc., Boulder, Colo.

Toxicity experiments. Increased toxicity to LPS in OF1 mice was assessed by simultaneous intravenous injection of the MDP derivative and LPS. Toxicity of serum from treated mice was evaluated in C3H/HeJ mice sensitized to TNF and IL-1 toxicity by an intraperitoneal injection of galactosamine (18 mg per mouse) given simultaneously with the intravenous challenge (30, 52). The number of survivors was recorded daily for 3 days, since no death occurred after that time. The 50% lethal dose (LD_{50}) was calculated by the method of Reed and Muench (45a). Survival rate was analyzed by the adjusted chi-square test.

Cytokine determination. Circulating levels of TNF, IL-1, IL-6, and IFN-γ in the sera of mice exsanguinated via the abdominal aorta at different times (1.5, 3, 6, or 12 h) after an intravenous injection of 10 μg of LPS were determined by bioassays and immunoenzymatic assays when available. Mice were primed with MDP or one of its derivatives by a single intravenous injection of 300 μg 3 h before the challenge with LPS. Three independent experiments were carried out with three or four mice for each point.

Bioassays were used to determine the level of TNF, IL-6, and IL-1 as previously reported (28, 29). Briefly, threefold dilutions of the test sample were applied in triplicate to L929α cells in the presence of actinomycin D. Cytotoxicity was determined colorimetrically after the remaining adherent cells were stained with crystal violet. The cytotoxic activity was defined as the reciprocal of the dilution resulting in 50% cytotoxicity on the regression line of log dilution against absorbance; 1 U of cytotoxic activity corresponds to about 5 pg of rMuTNF. IL-6 activity was measured by a [^{3}H]thymidine uptake assay with the IL-6-dependent hybridoma cell line 7TD1. Serum samples or recombinant IL-6 standard were serially diluted in triplicate. One unit was arbitrarily defined as the dilution producing half-maximal growth of the cells and was found to correspond to about 9 pg of rMuIL-6. IL-1 activity was determined by thymidine incorporation, using the D10S subclone of the D10.G.4.1 helper T-cell line as already reported (28). One unit corresponded to about 6 pg of rHuIL-1.

Immunoassays kits were used to determine TNF, IL-6, and IFN-γ activity. TNF and IL-6 levels in mouse serum were determined with enzyme-linked immunosorbent assay (ELISA) kits (Endogen Inc., Boston, Mass.) and IFN-γ levels were determined with murine ELISA kits (Genzyme, Cambridge, Mass.). Assays were performed exactly as described by the manufacturers, and each sample was determined in duplicate.

Measurement of IL-1 inhibitory activity. As in the bioassay of IL-1, the subclone D10S originally described by Orencole and Dinarello (37) was used in a cell proliferation assay. Cells (10^4 per well) were incubated in 96-well microplates with IL-1ra or with dilutions of serum samples for 1 h before addition of IL-1β as described previously (35). The plates were incubated for 3 days and then pulsed for 18 h with 1 μCi of [^{3}H]thymidine.

RESULTS

Sensitization to the lethal effect of LPS by muramyl peptides. We have observed that the LD_{50} of the *S. enteritidis* LPS in untreated mice was approximately 250 μg per mouse when the LPS was administered by the intravenous route. The influence of treatment with the various muramyl peptides was evaluated by using 25 or 50 μg of LPS, doses which have been used previously to establish the toxic synergism with MDP (8). Comparison between six synthetic muramyl peptides given at 100 or 300 μg per mouse shows that only two of them, one water-soluble MDP and one lipophilic MDP-GDP, produced a marked increase in LPS-induced death (Table 1). In such animals sensitized by muramyl peptide injection, death occurred on day 2 or 3 after LPS challenge; no mice died thereafter. In untreated mice given a lethal dose of LPS or in mice sensitized either by D-galactosamine administration or by adrenalectomy, the time course was shorter; most of the mice died within 24 h.

TABLE 1. Toxic synergism between MDP derivatives and LPS in female Swiss mice

Compound and amt (μg) in saline[a]	No. of mice dead/total no. after challenge with[b]:	
	25 μg of LPS	50 μg of LPS
Saline	0/12	0/24
MDP		
100		16/24**
300	8/12**	18/24**
Murabutide		
100		2/24
300	1/12	5/24
Murametide		
100		0/12
300	0/12	3/12
MDP-GDP		
100		10/24**
300	10/12**	20/24**
MDP(D,D)-GDP		
100		0/12
300	0/12	1/12
MDP(Thr)-GDP		
100		0/12
300	0/12	2/24

[a] Mice received muramyl peptides in saline intravenously at the same time as *S. enteritidis* LPS by the same route.

[b] Death recorded after 3 days. **, $P \leq 0.01$ versus saline pretreatment group by chi-square test.

Toxicity of sera obtained from mice treated with LPS and muramyl peptides. To determine the capacity to produce toxic cytokines in the various groups described in Table 1, the toxicity of sera in mice sensitized by galactosamine treatment was evaluated. To eliminate the effect of residual LPS in the blood, toxicity assays were performed with C3H/HeJ mice, a substrain known as a low responder to LPS. On the basis of data previously reported, we knew that in mice primed with MDP for enhanced TNF production following LPS challenge, the kinetics in the blood was not modified greatly even when high titers were obtained (43). Therefore, sera were collected 1.5 h after challenge with 25 μg of LPS in various groups of mice pretreated with BCG as controls or with different MDP derivatives. The highest intravenous dose of serum administered to the C3/HeJ mice was 200 μl, and mice were injected with twofold dilutions to determine an LD_{50} of each serum sample when possible. The results of toxicity tests and TNF levels in the various serum samples are shown in Table 2.

These results indicate that except in two groups pretreated with murabutide or murametide, sera from mice treated prior to LPS challenge contained elevated TNF levels and were toxic in galactosamine-sensitized C3H/HeJ mice. The estimated dose of TNF bioactivity found in the serum volume corresponding to 1 LD_{50} was about 30 ng for serum from mice pretreated with muramyl peptides. The toxicity of serum from BCG-infected mice was higher than expected on the basis of TNF content, but these animals are known to produce IFN-γ, which increases TNF toxicity (7, 50).

Thus, a relationship appears to exist between the level of bioactive TNF and the toxicity of mouse serum injected into galactosamine-sensitized recipients. However, comparison of these results with those reported in Table 1 indicates that a discrepancy can be seen between the priming effect for en-

TABLE 2. Toxicity of serum from mice challenged with LPS after pretreatment with muramyl peptides

Pretreatment[a]	TNF bioactivity (ng/ml) 1.5 h after LPS	Serum toxicity (LD_{50})[b]	
		Serum vol (μl)	Equivalent amt of TNF (ng)[c]
Saline	7	>200	
BCG	1,490	12.5	18.6
MDP	225	140	31.5
Murabutide	56	>200	
Murametide	8	>200	
MDP-GDP	337	76	25.6
MDP(D,D)-GDP	277	135	37.4
MDP(Thr)-GDP	244	148	36.1

[a] Mice received BCG organisms 2 weeks before the challenge with 25 μg of LPS or 300 μg of muramyl peptide by the intravenous route 3 h before LPS.
[b] LD_{50} was evaluated with three or four doses of twofold dilutions of serum (n = 10 or 15 per dose).
[c] In these assays, the LD_{50} of rMuTNF was 135 ng per mouse.

hanced TNF production and toxic synergism produced by some MDP derivatives. Further experiments were conducted to determine the serum cytokine profile at various time points after LPS challenge in mice pretreated with the various compounds.

Serum cytokine profile following LPS challenge in mice pretreated with muramyl peptides. Mice were given an intravenous injection of one of the six different muramyl peptides 3 h before a challenge with 10 μg of LPS. This amount of LPS was chosen because it is below the lethal dose when administered with MDP or MDP-GDP, which are the two compounds capable of increasing LPS toxicity (Table 1). Serum was collected at different time points after LPS challenge (1.5, 3, or 6 h) to determine the level of TNF, IL-1, and IL-6 bioactivity and of TNF, IL-6, and IFN-γ reactivity with ELISA kits in each serum sample. Serum samples from three or four mice were pooled in each group, and three independent experiments were performed.

Some of the data are reported in Table 3. Results are expressed as the level of cytokine determined in mice pretreated with MDP or MDP-GDP, two compounds which were found to be most potent as priming agents. The data reported here correspond to the level at the peak of the response for each cytokine. The peak was determined in preliminary assays by testing the level every 30 min after a challenge with the same type of LPS and in comparable mice, as reported in another study (29). None of the pretreatments modified the time course for the production of each cytokine compared with the response to LPS alone, even when the yield was greatly enhanced. When available, results obtained in both types of assay were compared, and they did not show large differences. As expected, mice pretreated with MDP or with the lipophilic derivative MDP-GDP produced substantial amounts of circulating TNF at 1.5 h after the LPS challenge. At the 3-h time point, the residual level of TNF was still detected in both groups; it was 15 and 24 ng/ml, respectively, in the bioassay (not reported in Table 3). IL-1 bioactivity was not detected at the 1.5-h time point, peaked at 3 h, and started declining thereafter. The level of IL-1 was increased (6- to 10-fold) after pretreatment with both muramyl peptides. Levels of IL-6 in serum were measurable by 1.5 h, peaked at 3 h, and then decreased; they remained around 15 to 25% of the maximum by 6 h before reaching background levels by 9 h. The release of IL-6 was potentiated (2.5- to 3-fold) by pretreatment with muramyl peptides. The production of circulating IFN-γ was detectable at low levels at the 3-h time point following administration of LPS alone. The levels were increased to a maximum at 6 h and were higher (twofold) in mice pretreated with MDP or MDP-GDP. The levels returned to baseline within 12 h (results not shown).

TABLE 3. Serum cytokine responses following LPS challenge in mice pretreated with MDP or MDP-GDP

Assay	Time (h) of bleeding[a]	Cytokine level (ng/ml)[b] after treatment with:			
		300 μg of MDP[c]		300 μg of MDP-GDP[c]	
		Bioassay	Test kit	Bioassay	Test kit
TNF	1.5	102 ± 11.9	92.3 ± 59	138 ± 39.4	166 ± 138
IL-1	3	0.83 ± 0.06	NT[d]	1.63 ± 0.7	NT
IL-6	3	633 ± 173	416 ± 127	604 ± 334	419 ± 22
IFN-γ	6	NT	20.1 ± 5.6	NT	20.3 ± 2.8

[a] Time after intravenous challenge with 10 μg of LPS.
[b] Level of cytokine in medium is given as the mean ± standard deviation from three independent experiments. In controls given LPS alone, the cytokine levels at the indicated times were 5 ± 6.4 ng of TNF, 0.14 ± 0.19 ng of IL-1, and 250 ± 50 ng of IL-6 in bioassays and 9.2 ± 1.3 ng of IFN-γ by ELISA kit.
[c] Intravenous injection 3 h before the challenge.
[d] NT, not tested.

To compare the influence of the various pretreatments on cytokine responses, results have been expressed as the mean percentage of the maximum level determined in each assay. The highest response in three identical experiments were obtained with MDP or MDP-GDP pretreatment (Table 3). Responses obtained with the other muramyl peptides indicate that except for murametide, all other compounds were able to prime mice for enhanced cytokine response. The time course of cytokine appearance was very similar in the various groups of mice, although the level in serum was sustained slightly longer when it was elevated at the peak of the response. In addition, and as seen in Fig. 1, the water-soluble murabutide, which does not increase LPS toxicity, was far less potent than MDP in increasing the cytokine levels. However, comparison of results obtained with mice pretreated with lipophilic MDP derivatives do not indicate that MDP(D,D)-GDP or MDP(Thr)-GDP, which did not modify LPS toxicity, were significantly less effective than MDP-GDP, with the exception of their effect on IL-6 production. This last result is surprising since both TNF and IL-1 levels were elevated in the sera of mice pretreated with MDP(D,D)-GDP or MDP(Thr)-GDP. In addition, it should be noted that an identical profile of cytokine responses was obtained when TNF and IL-6 levels were evaluated by immunoenzymatic assays (not reported in Fig. 1). These findings suggest that enhancement of LPS toxicity by a few muramyl peptides, namely, MDP and MDP-GDP, is hardly related to the level of a single cytokine and that compounds which did not increase LPS toxicity were generally less potent as priming agents for cytokine induction.

IL-1-inhibitory activity in mouse sera. Sera obtained in the experiments described above (Fig. 1) were also tested on D10S cells to evaluate their capacity to block the response to IL-1 in the bioassay. Modulation of IL-1 bioactivity was obtained with IL-1ra, which inhibits biological responses induced by IL-1 in vitro and in vivo (25) and improves the survival of mice when given after a lethal dose of LPS (1).

In the results reported in Table 3 and Fig. 1, IL-1 bioactivity peaked 3 h after LPS challenge and remained elevated at the 6-h time point in groups of mice given MDP or MDP-GDP before the challenge but also in animals pretreated with MDP(D,D)-GDP or MDP(Thr)-GDP. The same sera were exam-

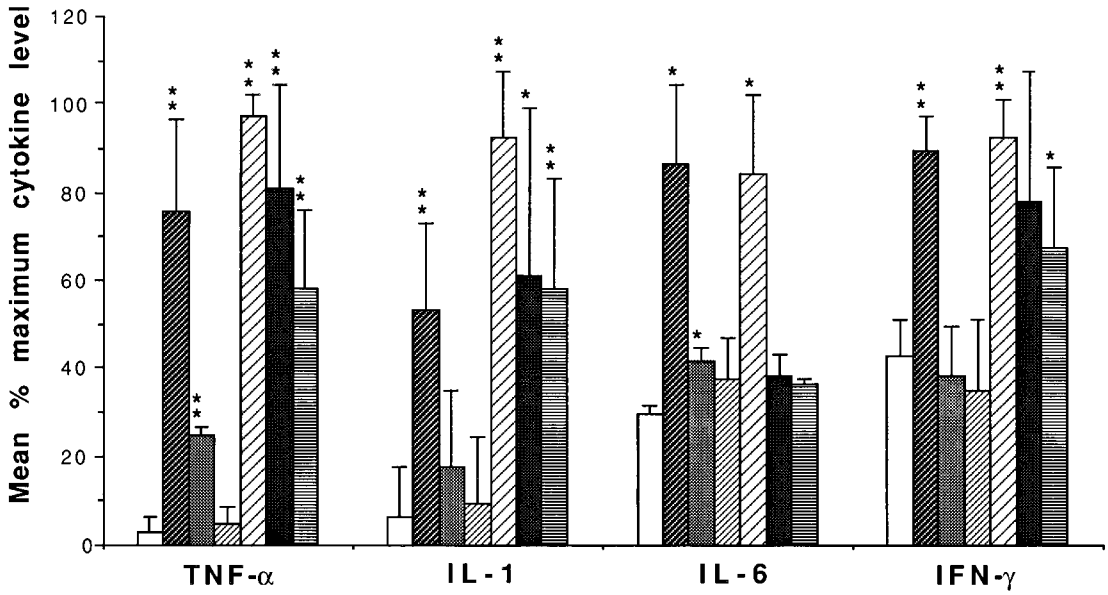

FIG. 1. Cytokine profile in mice following intravenous injection of 10 μg of LPS after intravenous pretreatment with saline or with the different muramyl peptides (300 μg per mouse). As in Table 3, we have presented results only at the time corresponding to the peak response for each cytokine (i.e., 1.5 h for TNF, 3 h for IL-1 and IL-6, and 6 h for IFN-γ. Results are expressed as the mean ± standard deviation of three separate experiments in bioassays for TNF, IL-1, and IL-6 and immunoassays for IFN-γ. Maximum levels (in nanograms per milliliter) obtained with MDP or MDP-GDP pretreatment are reported in Table 3. *, represents $P < 0.05$ and **, represents $P \leq 0.01$ versus saline pretreatment group by the Student t test. Symbols: □, saline; ▨, MDP; ▩, murabutide; ▨, murametide; ▨, MDP-GDP; ▩, MDP(D,D)-GDP; ▤, MDP(Thr)-GDP.

ined for their ability to inhibit the mitogenic effect of rHuIL-1, as has been demonstrated with IL-1ra (35). IL-1ra inhibited IL-1-induced proliferation in a dose-dependent manner, allowing us to determine in these assays a 50% effective dose which was 20 ng of IL-1ra against 0.01 ng of IL-1. Various dilutions of mouse serum were added to D10S cells under the same conditions before the addition of IL-1. A significant inhibition was found at the 3-h time point with serum from mice receiving their MDP injection before the LPS challenge, whereas it was less marked in the other groups. None of the sera used in the assays was cytotoxic for the cell line or displayed an inhibitory activity on IL-2 stimulation (data not shown).

Influence of water-soluble muramyl peptides on the mortality rate in galactosamine-treated mice challenged with LPS. Murametide and, to a lesser extent, murabutide have been shown to be poor priming agents for enhanced TNF production when given 3 h before the LPS challenge and not to enhance LPS toxicity (Table 1; Fig. 1). Both compounds were given before or after LPS in comparison with MDP to examine whether there was a possible beneficial effect. These experiments were conducted with galactosamine-treated mice known to be highly susceptible to the lethal effect of LPS or of TNF. When given 3 h prior to the challenge, MDP slightly increased the mortality rate in these animals and the two other analogs had only a marginal and insignificant protective effect. However, intravenous administration of murabutide or murametide 1 h after the intraperitoneal challenge resulted in significant protection (Table 4).

In a preliminary evaluation of the level of circulating TNF in these mice tested at 1.5 h following the challenge (41), it was found that when given after LPS, neither MDP nor murabutide significantly modified TNF production whereas murametide decreased the release of TNF into the blood by 30% (data not shown).

DISCUSSION

The findings presented in this study provide information on differences between synthetic compounds of the MDP series in their modulation of the LPS-induced responses. Thus, it has been confirmed that some muramyl peptides potentiated the lethal effect of LPS in mice whereas other, closely related compounds did not. Therefore, we failed to demonstrate a clear relationship between increased toxicity and production of circulating cytokines.

In considering the three water-soluble molecules used in this study, MDP, murabutide, and murametide, it appears that MDP, the only one which sensitized mice to LPS toxicity, was

TABLE 4. Effect of treatment with muramyl peptides on the incidence of mortality in mice challenged with LPS and D-galactosamine[a]

Treatment		Cumulative mortality (no. dead/total no.)	Protection (%)	P^b
Compound[c]	Time[d] of injection			
Saline	− 3 h	36/53		
MDP	− 3 h	46/53	0	
Murabutide	− 3 h	27/53	17	NS[e]
Murametide	− 3 h	29/53	13.2	NS
Saline	+ 1 h	37/54		
MDP	+ 1 h	15/26	10.8	NS
Murabutide	+ 1 h	14/54	42.6	0.01
Murametide	+ 1 h	9/35	42.8	0.01

[a] Mice were challenged intraperitoneally at 0 h with 18 mg of D-galactosamine and 50 ng of LPS (per mouse).
[b] Value obtained in the chi-square test.
[c] Compounds (300 μg) were administered via the intravenous route.
[d] Relative to time of challenge at 0 h.
[e] NS, not significant.

a potent priming agent for enhanced production of TNF, IL-1, IL-6, and IFN-γ, whereas the two others were ineffective or poorly active. The toxicity of the serum that was passively transferred to C3H/HeJ recipients treated with D-galactosamine correlated with the level of cytokines found in the blood. On the basis of the results obtained with the hydrophilic muramyl peptides, one could expect a strict correlation between enhanced cytokine production and sensitization to LPS toxicity. Pretreatment of mice with lipophilic compounds produced different patterns of response. Of the three analogs, only MDP-GDP potentiated the LPS-induced lethal effect, and it was powerful in stimulating cytokine production after LPS injection, being at least as active as MDP. However, the other two lipophilic compounds were effective in increasing the production of TNF, IL-1, and IFN-γ, and the toxicity of sera from mice treated with these compounds and LPS was comparable to that obtained with MDP-GDP. Unexpectedly, these two compounds were unable to increase IL-6 production despite the elevated level of TNF and IL-1, but the role of IL-6 in endotoxic shock is still controversial (33, 49, 53).

In summary, our studies indicate that immunological priming with the various muramyl peptides could produce different patterns of response to LPS lethality: sensitization, absence of sensitization, or even a protective effect as shown when murabutide or murametide was given after LPS administration. These responses to an LPS challenge appear to be independent of the cytokine levels, at least those measured in our study. The observation that only two of the four compounds able to prime cells for an enhanced cytokine production could increase LPS lethality may suggest a state of hypersusceptibility to LPS or to cytokines related to the production of other cytokines not determined in these groups, such as LIF (6) or migration inhibition factor (3, 7), or of secondary mediators, such as products of the arachidonic acid pathway (21, 26, 50) or of reactive nitrogen intermediates (23, 24, 38). In contrast to a study of mice primed with complete Freund's adjuvant (9), pretreatment with any of the muramyl peptides did not reduce monokine production below that obtained in controls given the LPS challenge alone. Therefore, a role in the absence of toxic synergism of mediators which reduce macrophage secretion directly, such as IL-4, IL-10, or transforming growth factor β (11, 16, 19, 51), or indirectly by binding to LPS such as serum lipoproteins (15, 32) appears unlikely. An increased production of soluble TNF receptors may still be involved as a protective mechanism against LPS lethality (2, 31, 36), although levels of the monokine were elevated. In our study, we did not find a demonstrable IL-1-inhibitory activity, suggesting the release of IL-1ra which could be responsible for a protective effect against LPS (1). A decrease in cell reactivity to LPS could be related to the down-regulation produced by some muramyl peptides in surface antigen expression (27, 47), namely, the CD14 molecule which functions as a receptor for LPS (reviewed in reference 54). A comparative study with several muramyl peptides is currently being performed to evaluate changes in surface markers of human monocytes.

The muramyl peptides used in our study can act as efficacious adjuvants in various animal models and can stimulate nonspecific resistance to infection (8, 39, 42, 48). An important difference is the capacity of MDP and MDP-GDP to induce a febrile response in the rabbit, whereas the four other compounds were inactive (40, 46). Some muramyl peptides do manifest neurobiological properties. Thus, a serotoninergic mechanism has been involved in somnogenic and pyrogenic effects of MDP (25, 34, 45); this mechanism could be considered to play a role in sensitization to LPS.

In conclusion, our data confirm that the role of cytokines in toxic shock is not yet fully understood and indicate that immunological priming could mediate harmful as well as beneficial activities. It would be particularly interesting to determine pathways involved in the increased incidence of survival in mice given an otherwise lethal dose of LPS when a muramyl peptide was injected after the challenge, inasmuch as it may be possible to extend the time window for treatment of potentially lethal endotoxemia. Finally, our study shows that a higher level of circulating cytokines did not correlate with LPS lethality. Moreover, the absence of toxic synergism was not related to a decrease in the production of one cytokine. Our results underline the importance of host resistance (or sensitization) to those potentially deleterious cytokines, particularly when associated with LPS (52). Other factors could be required for the induction of shock. Experiments are under way to evaluate the role of the several arachidonic acid metabolites after treatment with different muramyl peptides, since patients in septic shock have elevated levels of these lipids in their blood (26). In addition, we are examining the influence on induction of nitric oxide synthase of the synthetic adjuvants given either alone or combined with LPS. The nitric oxide overproduction is known to be involved in the pathogenesis of LPS-mediated shock, and differences between MDP analogs have already been reported in induction of nitric oxide production (38).

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